AD-A197 012

DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION
(U)

1b. RESTRICTIVE MARKINGS
NA

2a. SECURITY CLASSIFICATION AUTHORITY
NA

3. DISTRIBUTION / AVAILABILITY OF REPORT
Distribution unlimited

2b. DECLASSIFICATION / DOWNGRADING SCHEDULE
NA

4. PERFORMING ORGANIZATION REPORT NUMBER(S)
Wayne State University

5. MONITORING ORGANIZATION REPORT NUMBER(S)
NA

6a. NAME OF PERFORMING ORGANIZATION
Wayne State University

6b. OFFICE SYMBOL (If applicable)
NA

7a. NAME OF MONITORING ORGANIZATION
Office of Naval Research

6c. ADDRESS (City, State, and ZIP Code)
Dept of Physiology, Wayne State University
540 E. Canfield Ave, Detroit, MI 48201

7b. ADDRESS (City, State, and ZIP Code)
800 N. Quincy Street
Arlington, VA 22217-5000

8a. NAME OF FUNDING / SPONSORING ORGANIZATION
Office of Naval Research

8b. OFFICE SYMBOL (If applicable)
ONR

9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
N00014-87-K-0047

8c. ADDRESS (City, State, and ZIP Code)
800 N. Quincy Street
Arlington, VA 22217-5000

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO.
61153N	RR04108	441k702	

11. TITLE (Include Security Classification)
(U) Electrochemical Study of Phototransduction in Protein Pigment-Containing Model Membranes

12. PERSONAL AUTHOR(S)
Hong, Felix T.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 7/1/87 TO 6/30/88	1988 June 29	4

16. SUPPLEMENTARY NOTATION

17 COSATI CODES		
FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
membrane, pigment, integral protein, bacteriorhodopsin; rhodopsin; halorhodopsin; photoelectric signal; light-induced rapid charge separation; vision; photosynthesis.

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The objective of this project is to use primarily an electrochemical approach to study the fundamental molecular processes that underlie the light-mediated sensory (visual) and energy (photosynthetic) transduction in model retinal protein membranes. We take advantage of the chemical similarity between rhodopsin (visual pigment), bacteriorhodopsin (a light-driven proton pump) and halorhodopsin (a light-driven chloride pump) to explore possible common designs in these proteins for different functions.

We use a tunable voltage clamp method to analyze site-specific electric signals from membranes reconstituted from normal and chemically modified retinal proteins. We proposed a regulatory mechanism of visual transduction based on the early receptor potential. We also discovered site-specific signals from halorhodopsin membranes that are analogous to the B1 and the B2 component of bacteriorhodopsin membranes (named H1 and H2, respectively). Our preliminary results show that the H2 component is highly sensitive to aqueous chloride concentration.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS

21. ABSTRACT SECURITY CLASSIFICATION
(U)

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Dr. Igor Vodyanoy	(202)-696-4056	ONR

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

PROGRESS REPORT ON CONTRACT N00014-87-K-0047

PRINCIPAL INVESTIGATOR: Felix T. Hong Date: June 29, 1988

CONTRACTOR: Wayne State University

CONTRACT TITLE: Electrochemical Study of Phototransduction in Protein Pigment-Containing Model Membranes

START DATE: 1 December 1986

RESEARCH OBJECTIVE: The objective is to use primarily an electrochemical approach to study the fundamental molecular processes that under lie the physical basis of sensory transduction and energy transduction in retinal protein membranes.

PROGRESS (Year 2):

In this second year of project, we continued to investigate the pulsed light-induced photoelectric signals from reconstituted retinal protein membranes. We add to our repertoire halorhodopsin, a chloride transporting protein in Halobacterium halobium. Essentially, we were following the guidance of two of my theoretical papers (Publications #1 and #2). We treat active ion transport as a process with two interfacial charge transfer reactions coupled by a light-dependent step of ion pumping across the membrane. We have analyzed two experimental systems and have reached a generalization that the rate-determining step is the transmembrane step. As a consequence, we can treat the interfacial event independently of the intramembrane event. We have found two different electric signal originates from two surfaces of a bacteriorhodopsin membrane that have opposite pH dependence. The result suggests that the two signals reflect proton uptake and release, respectively, at opposite sides of the membrane. The implication is that we have three separate signals to probe the events at two surfaces and the event in the membrane interior (i.e., the signals are site-specific). This notion was reinforced by chemical modification studies with fluorescamine and N-bromoacetamide, showing that only the surface component is affected. We are now pursuing experiments with vitamin A analog substitution in order to gain a better insight into how it affects the photosignal. It also became apparent that site-directed mutagensis would be quite informative if it is coordinated and correlated with our site-specific electrical measurements.

A test of the generality of our approach is to apply it to a different retinal protein. Our recent experiment on halorhodopsin demonstrates the generality of this approach. The basic premise is that chloride ions must bind to or release from the surfaces in the light-induced pumping and that for every reaction there is also a reverse reaction. Therefore, we should be able to identify such a signal which has detectable dependence on the aqueous chloride concentration. As shown in Fig. 1, we observed two components: a positive-going signal (in the direction from extracellular to intracellular for positive charges, or in the opposite direction for negative charges). This signal is formally equivalent to B1 of bacteriorhodopsin and represents initial intramembrane charge separation (named H1). An additional component (H2) has opposite polarity is highly sensitive to chloride concentration. It can be reversibly inhibited by high chloride concentration. Tentatively, it is identified with the intracellular chloride release and its reverse reaction.

It is understandable to have two interfacial charge transfer reactions in an ion pumping membrane. It is harder to understand why there is a proton uptake in the sensory transducing pigment rhodopsin since there is no need to

transport proton across the membrane. Such a signal (the ERP R2 component) could serve an electrostatic regulatory function only if it occurs at the cytosol side of the rod outer segment disk membrane. In a lecture delivered at the Satellite Symposium on Molecular Electronics and Biocomputers in Budapest last August, I speculated about this possible mechanism. However, the crucial information about the side of proton uptake in a disk membrane was missing. This information was provided by M. A. Ostrovsky's recent work (Sensory Systems, Vol 1, pp. 117-126, 1987) . He demonstrated that the proton uptake is actually occurs at the cytosol side. Furthermore, there is no proton release at the intradisc side and that there is no transmembrane transport. We intend to follow this clue further. In the meantime, the available data up to this point suggest that there may be a common design in these retinal proteins although different functions are involved.

WORK PLAN (Year 3): We will continue to investigate the photoelectric effect of various reconstituted membranes with unmodified or modified retinal proteins. We will further characterize the chloride dependence of the H2 signal in halorhodopsin membranes. Whenever appropriate, equivalent experiments will be performed on bacteriorhodopsin as controls. For the halorhodopsin work, we are collaborating with Dr. Janos Lanyi, University of California, Irvine. Another collaborator, Dr. Richard Needleman, Wayne State University, Biochemistry Department, is working on genetic modification of halorhodopsin (site-directed mutagenesis). We are particularly interested in identifying chloride binding site(s). We are also collaborating with Dr. Hitoshi Shichi, Oakland University, on rhodopsin dry films. Our project on dielectric and metal coated thin film is making rather slow progress, mainly because our subcontractor was placing a low priority of our order. Recently, we are fortunate to find another supplier. The Electronics Design Center at Case Western University became interested in our work and is willing to manufacture the thin film assembly to our specifications at no costs. We are also seeking possibility of doing site-directed mutagenesis on bacteriorhodopsin via collaboration in order to fully exploit the site-specific nature of our photoelectric signal. If all goes well, we may want to place a greater emphasis on this aspect.

INVENTION: None

TRAINING ACTIVITIES

One postdoctoral fellow is working full time on the project. A high school student volunteer also worked during summer.

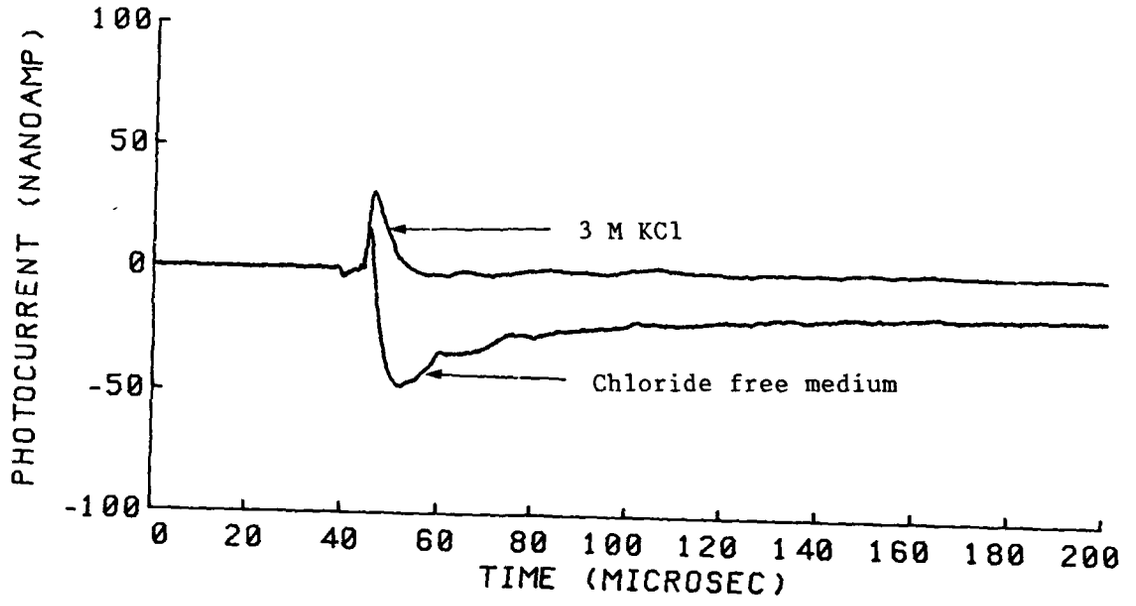

Fig. 1. Effect of varying chloride concentration on the fast photosignals from a reconstituted halorhodopsin membrane. The detailed methodology was described in Okajima and Hong (Biophys. J. 50:901, 1986). The photosignals are similar to those found in bacteriorhodopsin membrane. The B1 analog (named H1 component) has a positive polarity and the B2 analogy (named H2 component) has a negative polarity. The negative component is reversibly inhibited when chloride free medium is replaced by 3 M KCl. The apparent increase of the positive peak is probably a result of overlap of the two components rather than a real increase of H1 component. The explanation is similar to what we proposed for bacteriorhodopsin signals.

PUBLICATIONS:

1. Hong, F. T., Effect of Local Conditions on Heterogeneous Reactions in the Bacteriorhodopsin Membrane: An Electrochemical View, J. Electrochem. Soc., 134:3044-3052 (1987).
2. Hong, F. T., Internal Electric Fields Generated by Surface Charges and Induced by Visible Light in Bacteriorhodopsin Membranes, in Mechanistic Approaches to Interaction of Electric and Electromagnetic Fields with Living Systems, (M. Blank and E. Findl, Eds.), pp. 161-186, Plenum Press, New York (1987).
3. Hong, F. T. and Okajima, T. L., Rapid Light-Induced Charge Displacements in Bacteriorhodopsin Membranes: An Electrochemical and Electrophysiological Study, in Biophysical Studies of Retinal Proteins, (T. G. Ebrey, H. Frauenfelder, B. Honig, and K. Nakanishi, Eds.), pp. 188-198, University of Illinois Press, Urbana-Champaign (1987).
4. Hong, F. T., Electrochemical Evaluation of Various Membrane Reconstitution Techniques, in Proceeding of the 13th Annual Northeast Bioengineering Conference, March, 1987, Philadelphia, PA, (K. R. Foster, ed.), pp. 304-307, IEEE, Washington, D.C. (1987).
5. Hong, F. T., Effects and Roles of Internal Electric Fields in Pigment-Containing Biomembranes, in Proceedings of the Ninth Annual Conference of the IEEE Engineering in Medicine and Biology Society, November 13-17, 1987, Boston, Massachusetts, pp. 60-62, Institute of Electrical and Electronic Engineers, Inc., Washington, D. C. (1987).
6. Hong, F. T. and Conrad, M., The Bacteriorhodopsin Membrane as a Prototype Molecular Electronic Device, in Proceeding of the Third International Symposium on Molecular Electronic Devices, (F. L. Carter and H. Wohtjen, Eds.), Elsevier Science Publishers (North Holland), Amsterdam, in press (1988).
7. Hong, F. T., Interfacial Phenomena in Pigment-Containing Biomembranes, in Interfacial Phenomena in Biotechnology and Materials Processing, Boston, MA, August 3-7, 1987, (Y. A. Attia, B. M. Moudgil and S. Chander, Eds.), Elsevier Science Publishers, Amsterdam, in press (1988).
8. Hong, F. T., Control of Electric Signals in a Thin Film-Based Molecular Optoelectronic System, in Proceedings of the 10th Annual International Conference of IEEE Engineering in Medicine and Biology Society, New Orleans, LA, November 4-7, 1988, (G. Harris, Ed.), Institute of Electrical and Electronic Engineers, Inc., Washington, D.C., in press (1988).
9. Hong, F. T., Relevance of Light-Induced Charge Displacements in Molecular Electronics: Design Principles at the Supramolecular Level, Journal of Molecular Electronics, in press.

OTHER RELATED ACTIVITIES:

1. Organizer and Symposium Chairman, Symposium on Molecular Electronics - Biosensors and Biocomputers, 19th Annual Meeting of the Fine Particle Society, July 19-22, 1988, Santa Clara, CA.
2. Organizer of three sessions and Chairman of the Molecular Electronics Tract (1988-89), IEEE Engineering in Medicine and Biology Society 10th Annual International Conference, New Orleans, LA, November 4-7, 1988.

DISTRIBUTION LIST FOR REPORTS

ONR MEMBRANE ELECTROCHEMISTRY PROGRAM

Dr. Martin Blank
Department of Physiology
Columbia University College
of Physicians and Surgeons
630 W. 168th Street
New York, NY 10032

Dr. William E. Brownell
Department of Otolaryngology-HNS
Johns Hopkins University
School of Medicine
720 Rutland Avenue
Baltimore, MD 21205

Dr. Marco Colombini
Department of Zoology
University of Maryland
College Park, MD 20742

Dr. Michael A. Cusanovich
Department of Biochemistry
University of Arizona
Tuscon, AZ 85721

Dr. D. W. Deamer
Department of Zoology
University of California
Davis, CA 95616

Dr. Edward A. Dratz
Department of Chemistry
Montana State University
Bozeman, MT 59717

Dr. Harvey M. Fishman
Department of Physiology and
Biophysics
University of Texas Medical Branch
Galveston, TX 77550

Dr. Sol M. Gruner
Department of Physics
Jadwin Hall
Princeton University
P. O. Box 708
Princeton, NJ 08544

Dr. Felix T. Hong
Department of Physiology
Wayne State University
540 E. Canfield Avenue
Detroit, MI 48201

Dr. Huey W. Huang
Department of Physics
Rice University
Houston, TX 77251

Dr. Israel R. Miller
Department of Membrane Research
The Weizmann Institute of Science
Rehovot 76100
ISRAEL

Dr. V. Adrian Parsegian
Laboratory of Chemical Biology,
NIADDK
Room 9N-307
Building 10
Bethesda, MD 20892

Dr. Davis S. Perlin
Department of Biochemistry
Public Health Research Institute
455 First Avenue
New York, NY 10016

Dr. H. Gilbert Smith
EG & G Mason Research Institute
57 Union Street
Worcester, MA 01608

Dr. Michael E. Starzak
Department of Chemistry
State University of New York
Binghamton, NY 13901

Dr. H. Ti Tien
Department of Physiology
Membrane Biophysics Laboratory
Michigan State University
East Lansing, MI 48824

Dr. Tian Y. Tsong
Department of Biological Chemistry
Johns Hopkins University
School of Medicine
725 N. Wolfe Street
Baltimore, MD 21205

Dr. Peter Vanysek
Department of Chemistry
Northern Illinois University
De Kalb, IL 60115

ONR MEMBRANE ELECTROCHEMISTRY PROGRAM

Dr. Howard Wachtel
Dept. of Electrical & Computer Eng.
University of Colorado
Campus Box 425
Boulder, CO 80309

Dr. James C. Weaver
Div. Health Sciences & Technology
Room 20A-128
Massachusetts Institute of Tech.
Cambridge, MA 20742

Dr. George S. Wilson
Department of Chemistry
University of Kansas
Lawrence, KS 66045

Annual Final and Technical Reports

ADMINISTRATORS

Dr. Igor Vodyanoy, Code 1141SB (2 copies)
Scientific Officer, Biophysics
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Administrator (2 copies) (Enclose DTIC Form 50)
Defense Technical Information Center
Building 5, Cameron Station
Alexandria, VA 22314

Administrative Contracting Officer
ONR Resident Representative
(address varies - obtain from contract or
your business office)

Dr. Robert J. Nowak, Code 1113ES
Scientific Officer, Electrochemical
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Program Manager
Biological/Human Factors Division
Code 125
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

Program Manager Defense Technical
Support Technology Directorate
Office of Naval Technology, Code 223
800 N. Quincy Street
Arlington, VA 22217-5000

Annual and Final Reports Only (one copy each)

DoD ACTIVITIES

Commander
Chemical and Biological Sciences Division
Research Army Research Office, P. O. Box 1221
Research Triangle Park, NC 27709

Head
Biomolecular Engineering Branch
Code 6190
Naval Research Laboratory
Washington, DC 20375

Directorate of Life Sciences
Air Force Office of Scientific
Bolling Air Force Base Research
Washington, DC 20332

Final and Technical Reports Only

Director, Naval Research Laboratory (6 copies)
Attn: Technical Information Division, Code 2627
Washington, DC 20375